Lysostaphin

Week 6

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

After having transformed pSB1K3-T7-Lys in the previous week, protein expression was started this week. Gel purification of overhang PCR product with BamHI was performed so that the linker-tag sequence could be added in. And to ensure the correct gene expression, the products were sent for sequencing.

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1 IPTG-induced expression of pSB1K3-T7-Lys (Trial 1)

Responsible

Reskandi Rudjito and Bethel Tesfai Embaie

Protocols used

- Protein Expression

Experimental Set Up

Lys Samples	1.1, 1.2, 1.3, 2.1, 2.2, 2.3
IPTG Concentrations	0.5 mM and 1 mM

Each sample was either induced with 0.5 mM, 1 mM IPTG or uninduced (negative control). OD absorbance was measured every 15 minutes for 3 hours.

Results and Conclusions

No results at this stage. No growth; cells could have died when they were thawed.

Discussion and Troubleshooting

Unsuccessful expression of Lysostaphin. Re-run transformation in overnight liquid cultures for second trial of induction and sonication.

2 IPTG-induced expression of pSB1K3-T7-Lys (Trial 2) and Sonication

Responsible

Reskandi Rudjito and Bethel Tesfai Embaie

Protocols used

- QIAGEN MiniPrep
- Colony PCR
- Protein Expression
- Sonication

Modifications to protocols

- Re-picked colonies from transformed cells on LB agar. Picked 6 colonies (3 from each agar)
- PCR from the colonies using q5 polymerase
- Used the overnight culture as an inoculum for Lys expression
- Used the overnight culture to obtain plasmid
- Sonicated liquid cultures with 3 hours induction

Experimental Set Up

Overnight liquid cultures were prepared with 3 mL LB medium and 3 L Kanamycin. Colonies were re-picked from transformed cells on LB agar. Picked 6 colonies (3 from each agar)
Labelling: Lys 1.1, 1.2, 1.3, 2.1, 2.2, 2.3
Colony PCR performed using 12.5 l reaction mix with VF2 primers and Q5 polymerase.

Table 1: Colony PCR reaction mix

Component	12.5 l Reaction Volume μl Reaction	Final Concentration
5X Q5 Reaction Buffer	2.5	1X
10 mM dNTPs	0.25	200 M
10 M Forward Primer (VF2)	1	0.5 M
10 M Reverse Primer (VR)	1	0.5 M
Template DNA	0	<1 ng
Q5 High-Fidelity DNA Polymerase	0.125	0.02 U/l
Nuclease-Free Water	7.625	-

Plasmid Purification was performed using QIAGEN MiniPrep kit. The concentration of purified plasmid was determined.
Lysostaphin protein expression of samples Lys 1.2 and Lys 2.1 by 3 hour induction. (OD that was initially too high, was diluted with 1:2 of new LB + kanamycin).

Table 2: Nanodrop of purified plasmid

Sample	Nanodrop Concentrations (ng/L)
Lys 1.1	50.7
Lys 1.2	37.3
Lys 1.3	31.5
Lys 2.1	51.6
Lys 2.2	38.6
Lys 2.3	28.1

Table 3: Protein Expression Samples

Sample	OD at induction
Lys 1.2 Induced	0.6749
Lys 1.2 Uninduced	0.5866
Lys 2.1 Induced	0.5980
Lys 2.1 Uninduced	0.6159

Results and Conclusions

We ran a gel of colony PCR products that showed clear bands between 1000 and 1200 bp for all colonies but sample Lys 1.1 as shown in the Figure below.

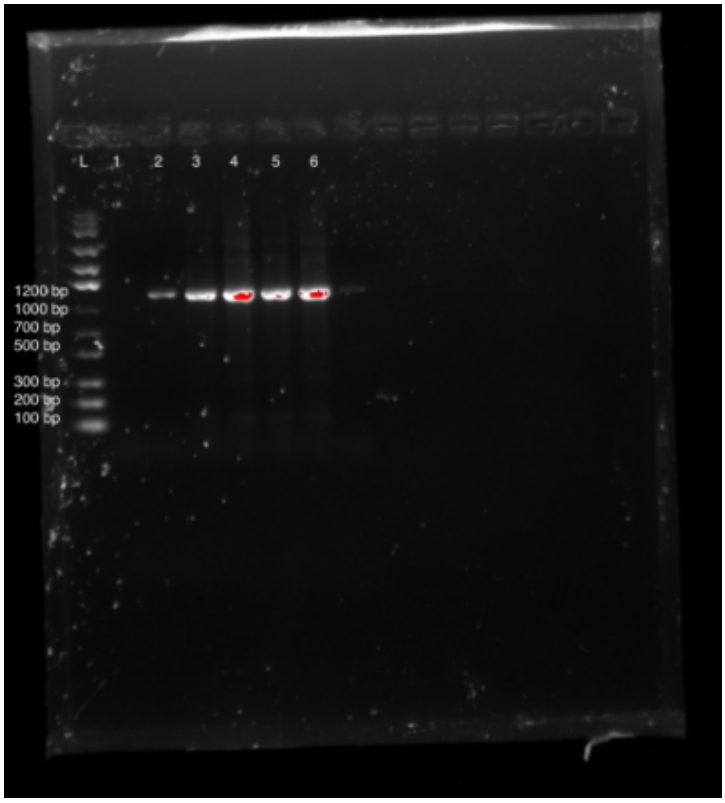

Figure 1: Colony PCR product of pSB1K3-T7-Lys. As previously labelled colonies:Lys 1.1, Lys 1.2, Lys 1.3, Lys 2.1, Lys 2.2, Lys 2.3, negative control

Lysostaphin expression and sonication were also successful.

Table 4: Lys Expression and Sonication

Sample	Pellet (in PBS) Conc. mg/ml	Supernatant (in Lysis Buffer) Conc. mg/ml
Lys 1.2 Induced	3	6.08
Lys 1.2 Uninduced	4.17	7.05
Lys 2.1 Induced	3	6.38
Lys 2.1 Uninduced	4.10	7.21

3 Sequencing of Lys

Responsible

Reskandi Rudjito

Experimental Set Up

Samples

Lys 1.1 (50.7 ng/μl)	90AA28
Lys 1.2 (37.3 ng/μl)	90AA32

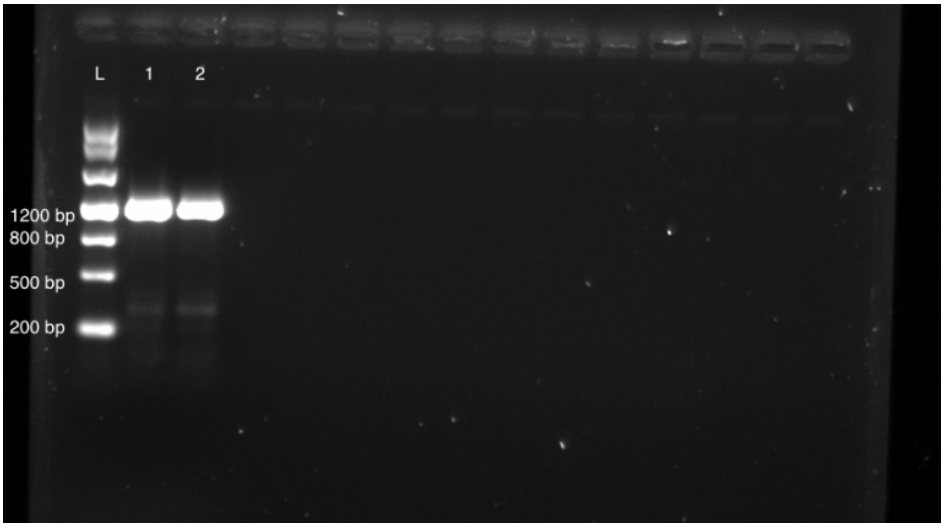

Figure 2: Gel of Colony PCR products Lys 1.1 and Lys 1.2 that were sent for sequencing

4 Gel purification of Overhang PCR product

Responsible

Shuangjia Xue, Oscar Frisell

Protocols used

- Gel Purification

Samples

Lys 3

Experimental Set Up

Each sample was the successful product of a Overhang PCR, with the BamHI that was inserted. The digestion and ligation of linker-tag sequence could start after Gel purification.

sample	10 ng/μl
loading dye	2.5 ng/μl
Total loaded vol	12.5 ng/μl

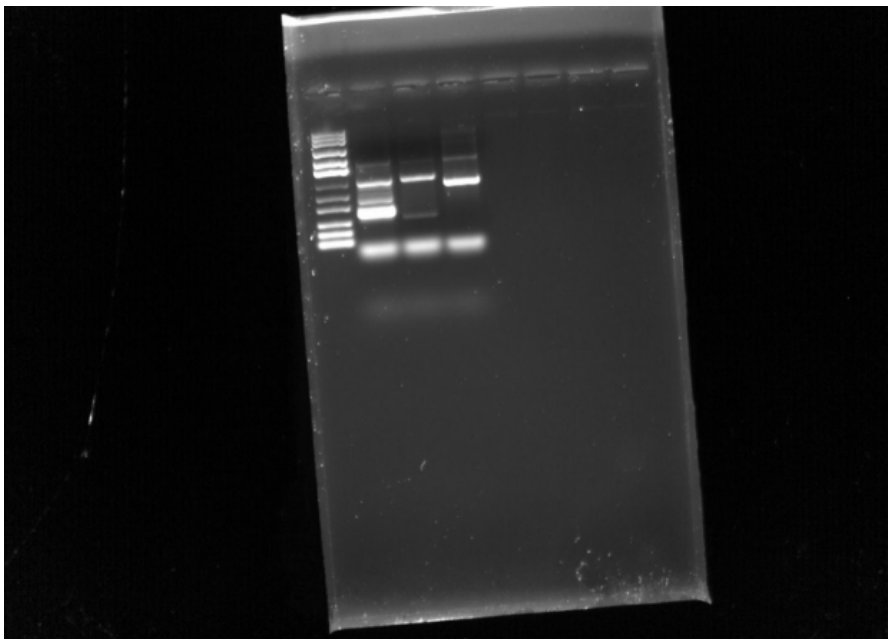

Figure 3: Lys well 3 - size 882bp